TOPICAL NANOEMULSION-BASED GEL OF ISOCONAZOLE NITRATE

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Abstract:

This study aimed to make an o/w nanoemulsion of isoconazole the drug nitrate (ISN) for topical use. Low aqueous solubility is a characteristic feature of the imidazole antifungal ISN. Therefore, ISN nanoemulsion would increase dispersibility and decreases skin resistance by enhancing the drug penetration to the first layers of skin (stratum corneum). The work included constructing the pseudo-ternary phase diagrams by using the aqueous titration method. The prepared o/w nanoemulsions were composed of oil, Smix (a mixture of surfactant and co-surfactant) and deionized water (DW). ISN nanoemulsions were subjected to characterization studies to choose the best formula. According to the characterization studies, the optimal formula, designated NE14 contains 1% ISN, 66% Smix ((1:3) tween60: propylene glycol:ethanol), 7% oleic acid, and 27% deionized water was reached. Formula NE14 is characterized by having a polydispersity index of (0.146), pH (5.76), droplet size (84.6 nm), percent transmittance (98.8%), viscosity (80m Pa.s) and a high release of isoconazole propably due low viscosity. The droplet size of NE14 (84.6nm) was also confirmed by an atomic force microscopy (AFM) research. The improved formula (ISN NE14) was found to be a promising nanoemulsion formula for enhancing the topical bioavailability of ISN and thus could increase its efficacy for the treatment of topical fungal infections.

Keywords: Nanoemulsion, Pseudoternary phase diagram, Isoconazole nitrate, Topical.

مستحلب النانو من نترات ايزوكونازول جل للاستخدام الموضعي

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الخلاصة:

كان الهدف من هذه الدراسة هو صنع مستحلب نانوي من دواء نترات ايزوكونازول (ISN)للاستخدام الموضعي. الذوبان المائي المنخفض هو سمة مميزة لـ ISN المضاد للفطريات، فإن مستحلب النانو ISN يزيد من قابلية التشتت ويقلل من مقاومة الجلد من خلال تعزيز تغلغل الدواء في الطبقات الأولى من الجلد (الطبقة القرنية). تضمن العمل إنشاء مخططات الطور الثلاثي الزائف باستخدام طريقة المعايرة المائية. تتكون مستحلبات النانو المحضرة من الزيت، smix) خليط من خافض للتوتر السطحي ومساعد خافض للتوتر السطحي) وماء مزدوج منزوع الأيونات (DDW) لاختيار أفضل صيغة، خضعت مستحلبات النانو إيزوكونازول لعدة دراسات توصيف. وفقًا لدراسات التوصيف، تم الوصول إلى الصيغة المثلى، المعنية NE14 ، والتي تحتوي على ١٪ ISN، 66٪ ((1: 3) Smix توين ٦٠: بروبيلين جليكول: إيثانول) ، ٧٪ حمض الأوليك ، و ٢٧٪ ماء منزوع الأيونات. تتميز الصيغة NE14 بوجود مؤشر تشتت متعدد قدره (٠,١٤٦) ، ودرجة الحموضة (٥,٧٦) ، وحجم القطرة (٨٤,٦ نانومتر) ، والنسبة المئوية للنفاذية (٩٨,٨٪) ، واللزوجة (٨٠ م باسكال) ، وتحرر الدواء مرتفع من أيزوكونازول بشكل مناسب بسبب اللزوجة المنخفضة. وقد تم تأكيد حجم قطيرة (84.6) NE14 نانومتر أيضًا بواسطة بحث مجهر القوة الذرية (AFM) .تم العثور على الصيغة المحسنة (ISN NE14) لتكون صيغة

مستحلب نانوي واعدة لتعزيز التوافر البيولوجي الموضعي للإيزوكونازول وبالتالي يمكن أن تزيد من فعاليتها في علاج الالتهابات الفطرية الموضعية.
الكلمات المفتاحية : مستحلب النانو ,مخطط الطورالثلاثي الزائف ,نترات الايزوكانازول ,للاستخدام الموضعي .

Introduction:

Topical drug delivery is one of the methods that has recently gained a large of interest for local, regional, and even systemic medication administration [1].When compared to the traditional oral dosing forms, topical formulations are superior and less harmful because It prevents stomach irritation caused by enzymatic activity and drug interactions with food , When the oral route is inappropriate, as in the case of vomiting and diarrhea, it can take the place of oral drug administration . In order to enhance the local and diminish the systemic effects or to make sure sufficient percutaneous absorption, The development of topical dosage forms aims to take advantage of drug delivery that allow for localization or penetration through the skin. [2]. Numerous studies have highlighted the pharmaceutical importance of nanotechnology as vehicles for dermal and transdermal delivery of a wide variety of drugs, since the physic-chemical properties of skin make difficult to some drugs penetration through the skin [3].

An advanced drug delivery technology, nanoemulsions are being evaluated for their potential to solve the problem of delivering such as drugs, biological active agents, and genetic materials that are problematic to their release [4] [5]. Oil has been emulsified in the appropriate ratio in aqueous phase surfactant and co surfactant to form a colloidal dispersion is called a nanoemulsion (NE). It has high kinetic and thermodynamic stability and is isotrpically clear.

ISN is 1-[(2RS)-2-[(2,6-Dichlorobenzyl) oxy]-2(2,4dichlorophenyl) ethyl]-lH-imidazole nitrate. ISN is an imidazole antifungal that works against a wide range of fungi, including Candida species, dermatophytes, and Malassezia furfur. And some gram positive bacteria can also be killed by it [6].

ISN belongs to class II of the Biopharmaceutics Classification System (BSC) (low solubility – high permeability)[7]. So Development of NE topical and transdermal drug delivery because the skin is the best site for medication administration. It has primarily been used to treat a number of skin conditions, including microbial and fungal infections[8].Such as the 1%w/w clotrimazole was prepared show better drug release, efficacy of drug penetration into the skin, higher in vitro retention time and increase antifungal activity compared to a commercial gel [9] [10] . An o/w nanoemulsion containing Itraconazole was formulated for transdermal application promising carrier for the transdermal delivery of drug for prolonged period along with reduced side effect, and offers a novel means for the treatment of fungal infections[11]. ISN cream (Travogen) containing 1% of the drug has been compared with ISN nanoemulsion gel showed that the gel better antifungal activity and drug penetration into the skin, in case of cream only 30% of dose found in skin while 90% of dose for gel [12].there is no previous study polished of ISN nanoemulsion .So the aim of study to prepare and evaluation of topical NE (<200nm) ISN oil in water (o/w) NE based Carbopol 934gel as topical dosage form to reduce resistance of drug by enhance penetration to first layers of skin (stratum corneum).

Materials and Methods:

Materials:

ISN, transcutol P, Triacetin was purchased from the Hyperchem Company in China. Tween (20,60,80), propylene glycol and

Oleic acid from Himedia Laboratories Pvt. in India. Ethanol was provided by Scharlau Laboratorie, Spain.

Methods:

Differential scanning calorimetry (DSC):

Using a Shimadzu 60 from Japan, for analysis, three to five milligrams of the pure drug (ISN) was stored in pans with aluminum seals. With nitrogen serving as a blank gas, sample heating was carried out for each set of samples at a rate of 10°C/min from 25 to 250°C [13].

Solubility studies:

ISN ability to dissolve in different oils (castor oil, liquid paraffin, oleic acid, Capmul PG8, Triacetin, Isopropyl mustard oil (IPM)), surfactants (tween 60, Triton X 100 and span 80) and co-surfactants (transcutol P, Cremophor, propylene glycol, and ethanol) was tested. A volume of 2 ml of different (oils, surfactants, and co-surfactants) were placed in small, plain tubes and then added to it an excessive amount of ISN powder. After that, the tubes were firmly sealed and kept at 25°C± 0.5 using a water bath shaker for 72 hours. The top layer of each sample was then filtered through a 0.45 m filter membrane after the samples had been spun at 3500 rpm for 20 minutes[14]. The samples were filtered and then diluted with methanol, and then the absorbance (eventually the solubility) was measured by UV-spectrophotometer at a λmax wavelength of the drug [15].

Development of pseudo-ternary phase diagrams:

The components of a pseudo-ternary phase diagram are oil, a combination of surfactant and co-surfactant known as "Smix," and deionized water (DW). These diagrams were made using the aqueous titration method [16]. Surfactant and co-surfactant were mixed together in different amounts (1:0.5, 1:1, 1:2, and 1:3). The oil to Smix ratio was mixed for each phase diagram in a variety of weight ratios until the best oil to Smix ratio was produced. There were twenty different oil and Smix combinations evaluated. Those mixtures were gradually titrated with DW and examined to determine whether they were clear. When oil in water (o/w) NE was formed (transparent and clear), the titration of DW stopped. No heat was used in the prepration of the formula. Pseudo-ternary phase diagram NEs do not contain any drugs in their composition. Thermodynamic stability tests were done on these NEs to find ways to make them more stable so they can be used to make ISN NEs [17].

Preparation of ISN loaded nanoemulsion:

ISN NEs were created by dissolving 1 gram of drug in a certain mixture amount of oil and co-surfactant then the determined amount of surfactant was added into the oil-co-surfactant loaded drug mixture, and using a vortex mixer the mixture was vortexed for 5 minutes. After that, the deionized water (the aqueous phase) was titrated slowly to create 100 gram of clear and transparent (oil in water) NEs were kept in sealed glass containers for further characterization studies [18] [19].

Characterization of ISN nanoemulsions:

The following characterization studies were performed:

Thermodynamic stability study:

Thermodynamic stability studies included the following tests:

- **Centrifugation test:** The mixtures of NEs that were prepared made were centrifuged at 3500 rpm for 30 minutes, and phase separation, creaming, and cracking were monitored through visual [17].

- **Heating- cooling cycle (H/C cycle):** The H/C cycle was used to investigate

the stability of NEs using temperature variation as a stress factor. Six cycles between 4°C and 45°C in the refrigerator, with no less than 48 hours of storage at each temperature were used. Then the Freeze - thaw cycle was used on formulations that are stable at these temperatures [20].

- **Freezing - thawing test:** The prepared NE formulations went through three cycles of freezing and thawing between -21°C and +25°C. At each temperature, they were kept for at least 48 hours. The formulas that passed these thermodynamic stress tests were used in further investigation [17].

Droplet size measurement and polydispersity index (PDI):
Using the dynamic light scattering technique, droplet size in NE was determined by observing the fluctuation in light scattering related to Brownian motion. Before measurement, NE was diluted 100 times with distilled water and gently mixed (to promote homogeneity) [21].

Percent of light transmittance (T %):
The percent of light transmittance was measured for ISN loaded NEs. The measurement was made by using UV-visible spectrophotometer at 650nm keeping distilled water as blank and by using quartz cuvette ,the % transmittance as a measure of optical clarity for the developed NE formulas was determined [22].

pH measurement:
The pH value of NE preparation is important, primarily to avoid skin irritation, and to maintain normal physiological PH. A digital pH meter is used to determine the pH of the formulations [23].

Viscosity measurement:
The samples viscosity was evaluated without dilution. The rheological properties of NE formulations were measured at 25±1°C using an NDJ-5S digital viscometer and spindle number (2) In a beaker, a viscosity spindle was put into 40 ml of prepared NE sample and revolved at varied speeds of 6, 12, 30, 60 and 100 rpm [24].

In- vitro release study:
The release of ISN from formulas was evaluated using dialysis-bag method (Molecular cut off 12000 Dalton "Da")[25]. Dissolution media (phosphate buffer with pH 7.4) was used to soak the dialysis bags. Each dialysis bag was filled with 1ml of NE formulation (equal to 10mg of ISN), which was then sealed at both ends and placed in a dissolution medium containing 150 ml phosphate buffer pH(7.4). The experiment was conducted in a USP dissolution apparatus type II (paddle method) with a 100 rpm rotation speed at 37±1^{0}C. At the specified times (10, 30, 60, 90, 120, 180, 240 and 300 minutes), 5 ml of the release medium was removed and replaced with a fresh phosphate buffer solution. UV-spectrophotometry at the drug λmax wavelength which is 273 that used to determine the concentration of ISN in the sample media at each of these time points by using the equation of calibration curve shown in Figure (1) [26] [27].

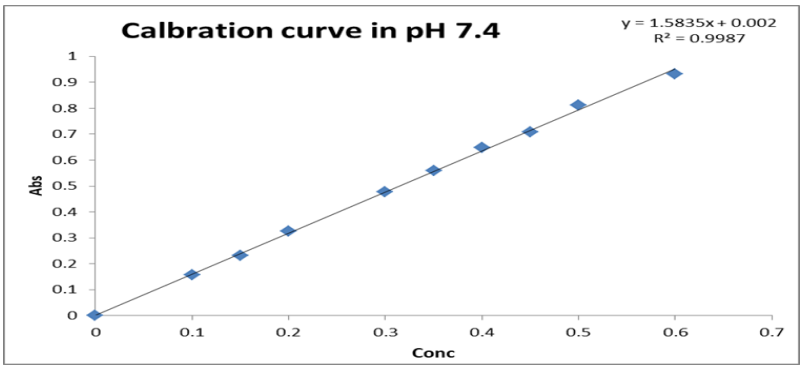


Figure (1): Calibration curve of ISN in 7.4 phosphate buffer (with 1% SLS).

Ex-vivo permeation study and skin deposition test:

For the ex vivo skin permeation study, six Franz diffusion cells with a water bath system device were used. There were lower donor and upper receptor compartments in each diffusion cell. The donor side of the Franz cell was in contact with the epidermal side of the skin at room temperature and sealed with an O-ring; the dermal side of the skin was exposed to the receptor media phosphate buffer (PBS) pH 7.4 with 1% Sodium Lauryl Sulphate (SLS) [28]. Following that, sample of ISN were applied to the donor compartment's skin's surface. In order to mimic in vivo conditions the receptor medium of PBS pH 7.4 / 1% (SLS) was stirred using a magnetic stirrer within the apparatus at 600 rpm during the experiment [29] [30]. Following that, 0.1 mL samples were frequently. The withdrawn samples were replaced with the same amount of fresh medium, Then using PBS / 1% SLS as a control, the withdrawn samples were subjected to spectrophotometric analysis at the ISN maximum absorbance [31].

At the end of 24 hours, the skin was separated and washed with DW. To extract the drug, it was cut into small pieces and subjected to a 30 minute sonication with methanol. The extract was then filtered through a 0.45-m syringe filter to determine the drug content present [32].

Drug - excipient compatibility study:

The following tests were used to

Fourier Transformed Infrared Spectroscopy:

The absence of interaction between formulation's components is a key feature of the produced NEs. FTIR spectroscopy explains that all excipients are compatible with the drug. The FTIR compatibility research was carried out by taking spectra of pure powdered ISN with a potassium bromide disk and of ISN NEs with a cuvette designed for liquid samples[17].

Atomic force microscopy (AFM):

The AFM is used to investigate the surface morphology of NE formulations which is capable of scanning surfaces in controlled environments. AFM is performed by AFMWorkshop, Nanotechnology research instrument that by diluting NEs in water and then placing the diluted NE onto a glass slide. The drops are then dried in an oven and scanned. We obtained a graph in three dimensions, a surface texture histogram, and a picture of the formula surface [33] [34].

Microbiological Study:

The anti-microbiological experiments were used to establish the effect of optimized ISN nanoemulogel formulations. The microbial effectiveness was evaluated using diffusion techniques (well diffusion technique). 1% ISN cream that presence in market, plain NE without drug, and the optimized formulation as ISN NE gel were inoculated onto sterile potato dextrose agar medium that had already been seeded with

the test organism *C.albicans*. Inoculation plates were then allowed to distribute the solution for 2 hours and incubated at 37°C for 24-48 hours. The inhibitory zone effect of the formulation was compared with that of the ISN cream and plain NE [35].

Skin irration test (Draize's test):
Draize's test for skin irritation was carried out with procedure described by John H. Draize *et al* (1944) using healthy Wister Albino male rats as a model design[36]. The visual observations were made every 1, 24 and 72hr for any signs of skin reactions or sensitivity like irritation, redness, edema, skin rash and erythema. The degree of skin irritation was assessed according to the mean score value obtained from both of erythema and edema observed or detected on the sample applied area of tested skin surface[37]. Draize dermal scoring criteria were used to evaluate the irritancy potential as described in Table (1).

Table 1: Draize dermal scoring criteria[37]**.**

P.I.I	Classification
0.0 - 0.4	No irritation
0.5 - 1.9	Slight irritation
2.0 - 4.9	Moderate irritation
5.0 - 8.0	Sever irritation

RESULTS AND DISCUSSION:

The Differential scanning calorimetry:
(DSC) theremogram of pure ISN showed in Figure (2). This thermo gram clearly showed sharp, an endothermic melting peak at 184.27 ° C. The peak was similar to the peak that was reported means that the drug was in its pure crystalline form [6].

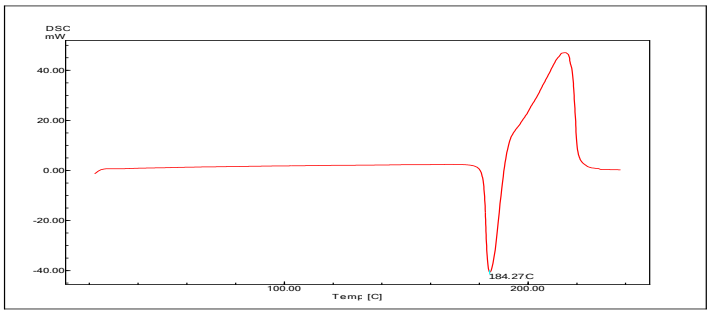


Figure (2): DSC Thermogram of ISN.

Saturation solubility study of ISN:
ISN saturation solubility in various oils, surfactants, and co-surfactants was determined as a significant step in the development of NEs since it enables choose the most convenient components for NE preparation and formulation and making a stable NE[38]**.**

The results of the solubility study of ISN in different oils, surfactants, and co-surfactants are all summarized in Table (1). Oleic acid was chosen as the oil phase, tween 60 as the surfactant, and transcutol p,PG and ethanol as the co-surfactant in the formulation of the NE. The different solubility of ISN due to different in oils structure and HLB.

Table (1): Saturated Solubility of ISN in different oils, surfactants, and co surfactants.

Oils	Saturated solubility (mg/ml)
Castor oil	1.32
Capmul PG8	2.656
IPM	11
Liquid paraffin	10.87
Oleic acid	20.96
Triacetin	6.11
Surfactants	**Saturated solubility (mg/ml)**
Span 80	1.6
Tween60	10.92
Triton X 100	2.5
Co-surfactants	**Saturated solubility (mg/ml)**
Cremophor	12
Ethanol	38.34
PG	40.84
Transcutol p	35.76

A pseudo-ternary phase diagram consists of three components: (oil, DW, and S mix (surfactant:co-surfactant)), the latter of which is a variable because its ratio can vary. Which is a variable part because it can have different ratios, such as (1:0.5, 1:1, 1:2 and 1:3). Figure (3 and 4) shows these different ratios. The area of NE is represented by the shaded region, and a bigger shaded region indicates good Nano-emulsifying activity.The different solubility of ISN due to different in oils structure and HLB.

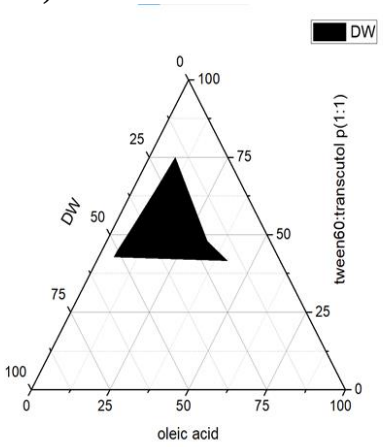


Figure (3): Pseudo ternary phase diagram of oleic acid, tween60, transcutol P, and DDW at Smix ratio (1:1).

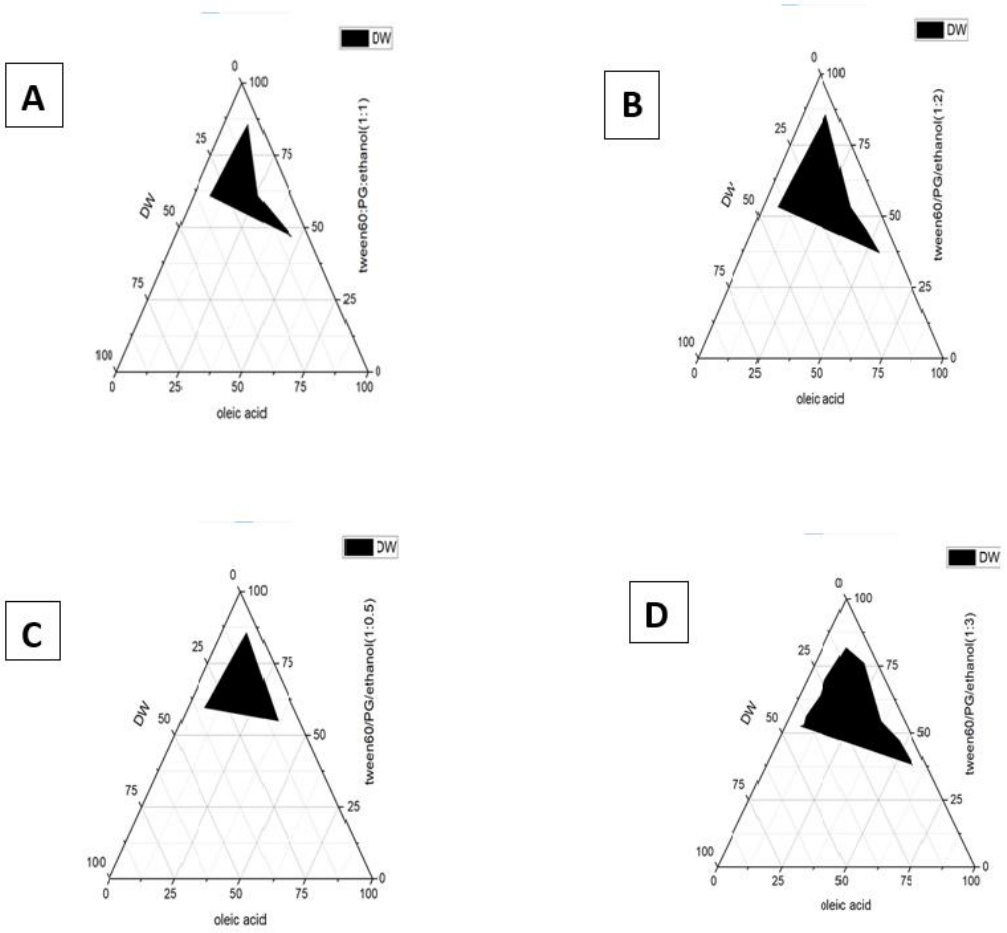


Figure (4): Pseudo ternary phase diagram of oleic acid, tween60, PG and ethanol, and DDW at Smix ratio: (A) 1:1 (B) 1:2 (C) 1:0.5 (D) 1:3.

Thermodynamic stability study:

Thermodynamic stability experiments were performed on formulations that were developed using a pseudo-ternary phase diagram, and the results were summarized in Table (2).by using blank emulsions Thermodynamic stability tests were done on these NEs to find ways to make them more stable so they could be used to make ISN NEs.

Depending on the findings of tests for thermodynamic stability considering a large percentage of water, a low ratio of S mix, no signs of phase separation, cracking, or a change in smell or color were observed. as in Evaluation Antifungal NE Using Posaconazole results showed no signs of instability like that (phase separation, creaming, and cracking) [35].Fifteen different formulas were chosen to create ISN-loaded NEs, which then they were subjected for further characterization [39]. The creaming or sedimentation rate can be increased by centrifugation, which shows that the emulsion breakdown can be related to gravitational force action. Owing to lower oil droplet density relative to aqueous media, the emulsion system of the O/W usually experiences creaming instead of the sedimentation[40].

Table (2): Thermodynamic stability parameters of ISN NE.

S mix ratio	no. of Formula	component nanoemulsions (W/W %)				Thermodynamic stability study			Result
		S mix (Surfactant:CoSurfactant) Tween60 as surfactant		Oil phase	DDW	Centrifuge	Freeze and thawing	Heating and cooling	
1:1	F-1	70(35:35)	PG+ ethanol	10	20	√	√	√	PASS
	F-2	75(37.5:37.5)	PG+ ethanol	10	15	√	√	√	PASS
	F-3	67(33.5:33.2)	PG+ ethanol	13	20	√	√	√	PASS
	F-4	60(30:30)	PG+ ethanol	15	25	_	_	_	FAILED
	F-5	60(30:30)	transcutol p	10	30	√	√	√	PASS
	F-6	55(27.5:27.5)	transcutol p	10	35	√	√	√	PASS
1:2	F-7	70(23.4:46.6)	PG+ ethanol	8	22	√	√	√	PASS
	F-8	66(22:44)	PG+ ethanol	7	27	√	√	√	PASS
	F-9	67(22.4:44.6)	PG+ ethanol	13	20	√	√	√	PASS
	F-10	80(26.6:53.4)	PG+ ethanol	9	11	√	√	√	PASS
	F-11	69(23:46)	PG+ ethanol	6	25	√	√	√	PASS
1:3	F-12	68(17:51)	PG+ ethanol	5	27	√	√	√	PASS
	F-13	69(17.25:51.75)	PG+ ethanol	6	25	√	√	√	PASS
	F-14	66(16.5:49.5)	PG+ ethanol	7	27	√	√	√	PASS
	F-15	68(17:51)	PG+ ethanol	10	22	√	√	√	PASS
	F-16	65(16.25:48.75)	PG+ ethanol	10	25	_	_	_	FAILED
	F-17	64(16:48)	PG+ ethanol	11	25	√	√	√	PASS
1:0.5	F-18	75(50:25)	PG+ ethanol	5	20	√	_	_	FAILED
	F-19	70(46.6:23.4)	PG+ ethanol	5	25	_	_	_	FAILED
	F-20	65(43.3:21.7)	PG+ ethanol	15	20	_	_	_	FAILED

Preparation of ISN NEs:

1 gram of ISN was dissolved in the appropriate amount of an oil and S mix to produce 100-gram NEs formulae. For the purpose of studying the characteristics of prepared NEs, several experiments were done.

According to the results, Upon completion of the thermodynamic stability studies , The prepared NEs that showed no signs of instability like that (phase separation, creaming and cracking) [40].

Characterization of ISN NEs:

For the purpose of studying the characteristics of prepared NEs, several experiments were done. The measurements of droplet size, PDI, Percent of light transmittance (T%) and pH value for ISN-loaded NEs (NE1–NE15) were shown In the Table (3and 4).

Table (3): particle size (nm) and PDI of ISN NEs.

no. of Formula	Droplate size(nm)	PDI
F1	44.9	0.269
F2	156.6	0.402
F3	180	0.385
F5	122.8	0.2601
F6	174	0.345
F7	202	0.4489
F8	261	0.516
F9	402	0.8062
F10	69.3	0.2606
F11	142.8	0.392
F12	190.7	0.237
F13	212	0.486
F14	84.6	0.146
F15	117.7	0.288
F17	112.5	0.115

The optimum particle size of the prepared NE formulas that have been below 200 nm, as was mentioned in Table (3), because the stability behavior of NE has markedly affected by the size of the obtained droplets, it also governs the rate and extent of drug liberation and absorption. In prepration of Rizatriptan Benzoate NEit was discovered that NEs with droplets smaller than 200 nm have some retention and may penetrate the skin [24]. So eleven formulas (F-1, F-2, F-3, F-5, F-6, F-10, F-11, F-12, F-14, F-15, and F-17) were chosen for further studies.

Table (4): Characterization of ISN NEs: T% and PH value (Mean± SD, N= 3).

no. of Formula	T%	pH
F-1	96.5±0.22	4.88±0.01
F-2	97.2±0.3	5.21±0.04
F-3	97.3±0.02	5.01±0.05
F-5	96.8±0.11	5.34±0.02
F-6	97.8±0.01	5.01±0.04
F-10	96.9±0.05	5.99±0.02
F-11	97.1±0.07	5.06±0.03
F-12	97.4±0.21	4.9±0.03
F-14	98.8±0.08	5.76±0.02
F-15	95.8±0.12	5.06±0.03
F-17	96.8±0.08	5.14±0.01

Analysis of the characteristics (pH value, droplet size, %T, and PDI) of ISN NEs showed that the size of the droplets decreased as the S mix ratio increased for 1:1 (NE1-NE6), 1:2 (NE7-NE11), and 1:3 (NE1-NE6) (NE 12- NE 17). Also, analysis of formulations showed that there were strong links between the size of the droplets in these NEs and the amount of S mix (w/w %). if the amount of oil increased, the size of the globule would also increase as in F3 and F9. As the results showed in prepration of Rizatriptan Benzoate NE that the particle size decreased as the surfactant concentration increased because a high surfactant concentration during homogenization reduces surface tension and stabilizes newly created surfaces[41].

The (PDI) measurement provides information on the droplet size homogeneity within the prepared NE, A PDI value that ranges from 0.0 to 1.0. The lower value has homogeneity of the particles in the formulation from the higher PDI [21].The PDI results showed that the droplets were evenly dispersed across the various formulations. The PDI for all formulations were less than (1.0).Since PDI for NE14 was 0.146 , its high uniform droplet distribution within the formulation is reflected in that value, due to lower particle size and lower Smix value [42].

The pH measurements of both formulas (drug-free and drug-loaded) show that oleic acid which was used as the oil phase in both formulations made the pH values of the drug-free NEs slightly acidic. The pH of the ISN-loaded NEs was elevated when the basic drug ISN was added to drug-free NEs. For NE14, the pH of the ISN NEs formulation was (5.76). The measured pH values of NEs were suitable for topical application due to comparable values with the skin pH, which ranges from 4.5 to 6.5 and therefore evade skin irritation and/or sensitivity [43].

The results of T% demonstrate that the produced NEs were transparent and clear, and Table (4) shown NEs' transparency. The ISN NE with the highest T% was NE14 (98.8%).

Viscosity Measurement:

The results of ISN NEs viscosity were observed to be in the range (26.00 – 150.00 mPa.sec.). All ISN NEs were found to have a low viscosity results, exhibit free

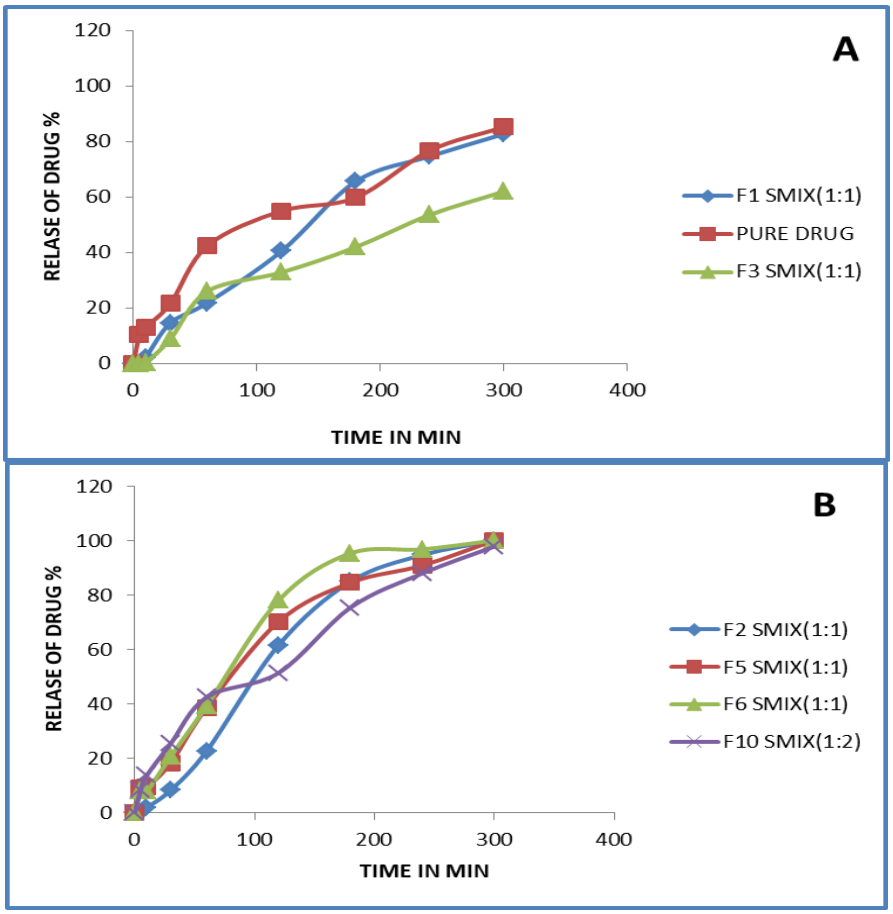
flowing with decreased viscosity that enable easy of spreading without washout or drug loss upon its application on skin surface [44]. When the concentration of surfactant increased, such as in F1and F2, the viscosity raised. Also the viscosity measurements revealed that as the ethanol concentration was increased, the viscosity of the ISN loaded NE formulations decreased [45].

In vitro release of ISN NEs study:

The release of the most of prepared ISN NE formulations were about 100% at the end of 300 min. A comparison between the dissolution profiles of ISN from different NE formulas was made using *f2*. According to the FDA guidelines, the similarity of the dissolution profiles occurring when values of *f2* values greater than 50, and dissimilarity value less than 50.

The release profile from F1 NEs formula which had high viscosity were slow release, it has un similar release, this may be attributed to the higher viscosity of all NEs [24].

Also release profile for F3 NEs formula showed that the release of ISN was lower from formula which has higher oil concentration, and with low Tween 60 content in the formula, which resulted in low ISN diffusion from the dialysis bag to the dissolution medium [46] [24].



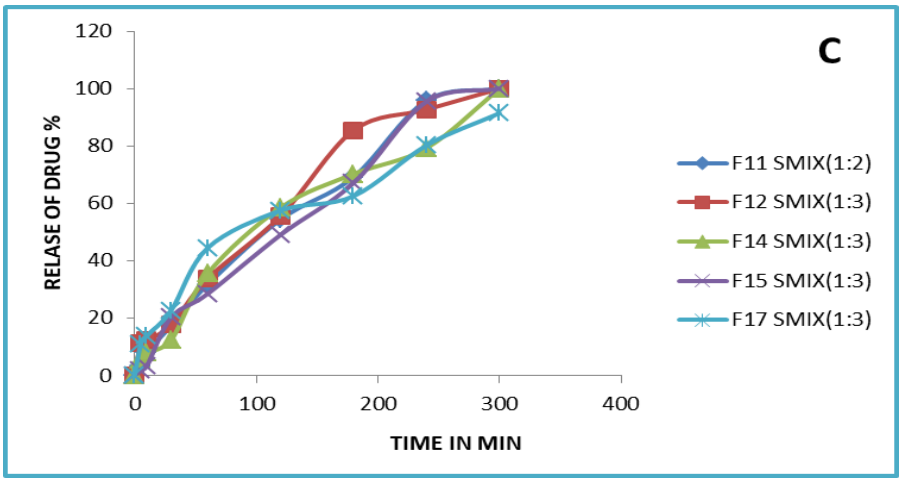


Figure (8): Release of ISN NEs: (A) release from (NE1 and NE3) (B) release from (NE 2, 5, 6 and NE10) (C) release from (NE11, 12, 14, 15 and NE17).

Selection of the best formula of ISN NEs was done after studied the properties of the ISN NEs that were made, there was an indication that (NE14) is the best formula because it gave droplets that were good in size (84.6 nm), good pH value for topical use (5.76±0.02), best percent transmittance (98.8%±0.08), accepted viscosity range (80±0.22 m Pa.s), low PDI (0.146) and higher release of ISN. The optimized formula might be further characterized. As result found in Selection of optimized formula of nimodipine NE which undergo for further characterized including FTIR, DSC and SPM[17].

Ex-vivo permeation study and skin deposition test:

Permeation rates of the drug were calculated over time as shown in Figure (9-A). After 6-hour found that drug penetration was less than 10% across the skin for formula and control. The NE was created with the purpose of increasing drug deposition through the first layers of the skin.

According to the results, after 24hr the drug deposition in skin was almost increased in NE compared to control as shown in Figure(9-B)[32].

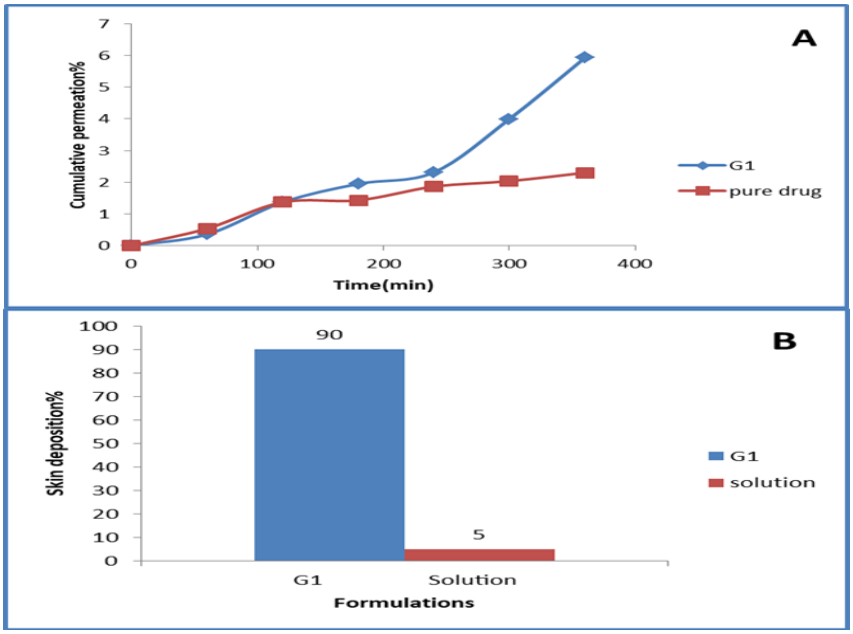


Figure (9): Skin permeation studies: (A) Skin permeation study (B) Skin deposition study.

Drug and excipient compatibility study: Fourier Transformed Infrared Spectroscopy:

For pure drug ISN and the ISN (NE14), FTIR spectra were made. The characteristic peaks of pure ISN drug shown in Table (5).

Table (5): characteristic peaks of pure ISN.

Bands (cm^{-1})	Interpretation
1621	C=C stretching of benzene ring
1581.4 and 1562.9	C=N bond stretching of aromatic rings
1253.13	C-O-C stretching vibration
1501	Stretching vibration of CH2 in Benzene
2952.24 and 2993.31	Stretching vibration of CH in Benzene

These peaks of pure ISN can be seen in the spectrum of ISN NE, as shown in Figure (9). There was also a broad peak from 2972.83cm^{-1} to 3363cm^{-1}. This broad peak comes from the OH- stretching of oleic acid, which occurs when oleic acid and water form hydrogen bonds. This shows that all of the components of the prepared NEs were compatible with both the drug and the excipient[17].

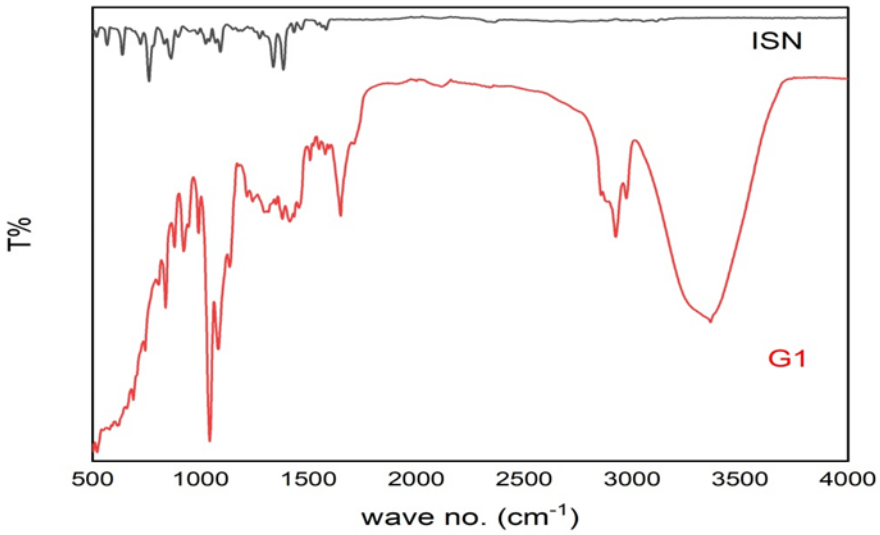


Figure (9): FTIR of pure ISN and NE gel of F14.

The FTIR spectra of carbopol 934p, PG, ethanol, tween60, and oleic acid showed in Figure (10) respectively. The selected formulas displayed the same functional groups band with some of stretching frequencies shifting because of the solubilization of ISN in formulas[47].

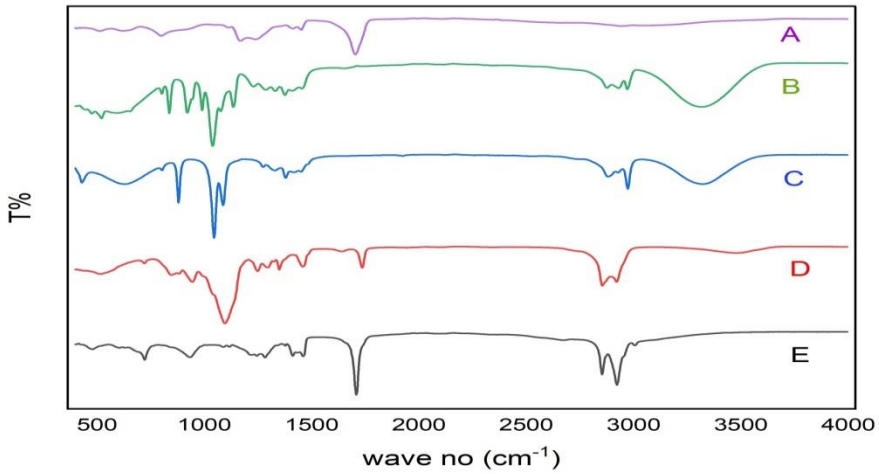


Figure (10): FTIR of excipient include: (A) carpobol 934 (B) PG (C) ethanol (D) tween60 (E) oleic acid.

Atomic force microscopy (AFM):

Atomic force microscopy could be used to characterize the shape, morphology, and surface structure of the samples being evaluated. The shape of the droplets was approximately spherical, with a smooth surface as shown in Figure (11). The histogram was found to be regular as it gradually declines; the graph resembles a bell curve [48] [49] . The formula analysis produced a particle size distribution with particles ranging in size from 70 nm to 10 nm. The mode of the particle size distribution occurred between 10 and 15nm, indicating that the distribution was skewed toward the smaller end of the scale[50].

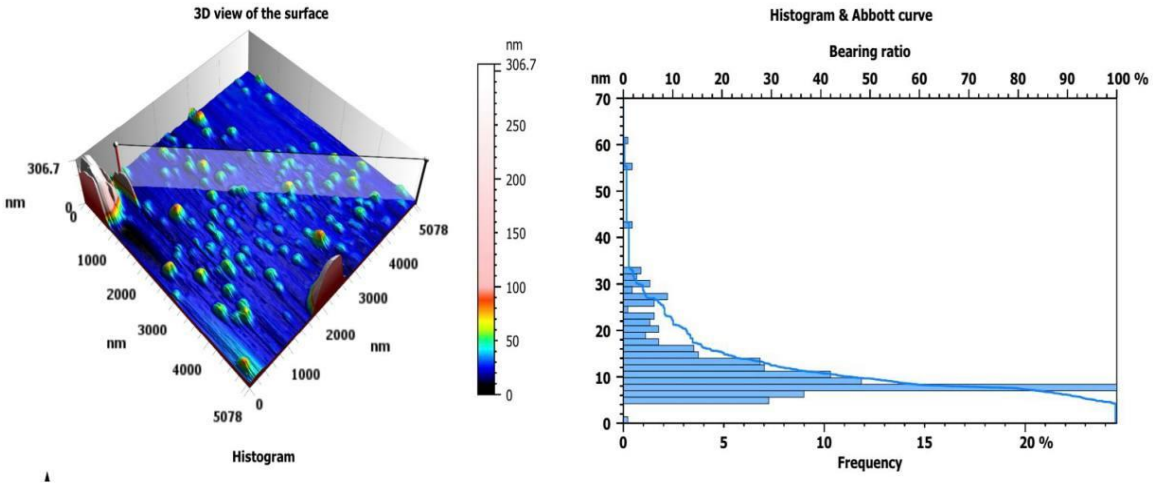


Figure (11): Histogram of surface texture of ISN NE gel by AFM.

Microbiological Study:

For determined the zone of inhibition, the *in vitro* antifungal inhibitory activity of 1% ISN NE gel (F14), 1% ISN cream, and plain NE gel was evaluated on the organism *Candida albicans*. The values for the zone of inhibition are shown in Table (6).

The results of the comparison between the inhibition zone values of the test and the inhibition zone values of the two controls showed that the F14 formula had the highest inhibition zone, as shown in the Figure (12). This could be due to ISN was expressed in a soluble form that allowed it to easily penetrate and effect fungal growth [51].

Table (6): Antifungal activity of 1% ISN NE gel (F14), 1% ISN cream and plain NE gel represented by inhibition zone in (mm).

Organisms	Inhibition zone (mm)		
	F14	1% ISN cream	Plain NE
Candida albicans	**40 ±1.2**	**30 ±0.95**	**0**

Figure (12): Inhibition zone of: (1) plain (2) 1% ISN cream (3) ISN NE gel F14.

Skin irritation study (Draize's test):
To confirm the safety of prepared F-14 ISN o/w NE gel, skin irritation test was done for selected formulation F14 after topical gel formulation, in which tested sample demonstrated mean score value of zero. Consequently, non-irritant topical application was achieved of the formulated F14 ISN o/w NE gel to skin, in addition to the safe and compatible employment of the ISN NE gel components on the skin[40].

Figure (13): Skin irritation test results on Wistar rat dorsal skin.

Conclusion:

Based on the results of this study, it can be concluded that the oleic acid, Tween 60, ethanol, and PG used to make the ISN topical NE formulation worked well. The results showed that the prepared formula was more effective for fungal activity. Based on physicochemical parameters and ex vivo data studies, it may be concluded that the developed NE system can be a promising carrier for the topical delivery of ISN with fewer side effects and offers a new method for treating fungal infections like C.albicans.

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